

INTERNATIONAL SEARCH REPORT

*including
References Cited.*

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 99475 WO	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/EP 00/07694	International filing date (day/month/year) 07/08/2000	(Earliest) Priority Date (day/month/year) 13/08/1999
Applicant AKZO NOBEL N.V.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

USE OF CHEMICAL CHELATORS AS REVERSAL AGENTS FOR DRUG-INDUCED NEUROMUSCULAR BLOCK

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

INTERNATIONAL SEARCH REPORT

Information on patent family member

International Application No

PCT/EP 00/07694

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
AU 3662895 A	09-05-1996	AU 708107 B	29-07-1999

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/07694

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/724 A61K31/194 A61P39/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	B DESIRE: "Inactivation of sarin and soman by cyclodextrins in vitro" EXPERIENTIA, vol. 43, no. 4, 1987, pages 395-397, XP000907287 the whole document ---	1-5,8,11
X	B. DESIRE: "Interaction of soman with beta-cyclodextrin" FUNDAMENTAL AND APPLIED TOXICOLOGY, vol. 7, no. 4, 1986, pages 647-657, XP000911170 the whole document ---	1-5,8,11
Y	AU 36628 95 A (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 9 May 1996 (1996-05-09) the whole document ---	1-5,8-11
-/--		

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

8 document member of the same patent family

Date of the actual completion of the international search

21 May 2001

Date of mailing of the international search report

20/07/2001

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/07694

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	C. MAY: "Development of a toxin-binding agent as a treatment for tunicamycinuracil toxicity: protection against tunicamycin poisoning of sheep" AUSTRALIAN VETERINARY JOURNAL, vol. 76, no. 11, 1998, pages 752-756, XP000913500 the whole document --- <i>Uekama</i> ---	1-5,8-11
Y	K. UEMA: "Effects of cyclodextrins on chlorpromazine-induced haemolysis and nervous systems responses" J. PHARM. PHARMACOL., vol. 33, no. 11, 1981, pages 707-710, XP000911185 the whole document ---	1-5,8-11
Y	T. IRIE: "Protective mechanism of beta-cyclodextrin for the hemolysis induced with phenothiazine neuroleptics in vitro" J. PHARMACOBIO-DYNAMICS, vol. 6, no. 6, 1983, pages 408-414, XP000911177 the whole document -----	1-5,8-11

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 18 April 2001 (18.04.01)	
International application No. PCT/EP00/07694	Applicant's or agent's file reference 99475 WO
International filing date (day/month/year) 07 August 2000 (07.08.00)	Priority date (day/month/year) 13 August 1999 (13.08.99)
Applicant BOM, Antonius, Helena, Adolf et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

02 March 2001 (02.03.01)

☐ in a notice effecting later election filed with the International Bureau on:2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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(19) World Intellectual Property Organization
International Bureau



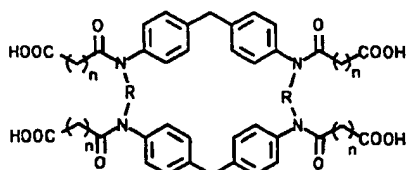
(43) International Publication Date
22 February 2001 (22.02.2001)

PCT

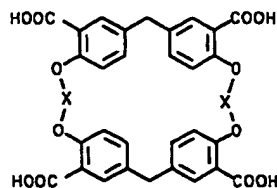
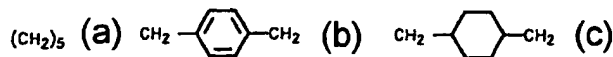
(10) International Publication Number
WO 01/12202 A2

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- (21) International Application Number: **PCT/EP00/07694**
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- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
99306411.2 13 August 1999 (13.08.1999) EP
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- (75) Inventors/Applicants (for US only): **BOM, Antonius, Helena, Adolf** [NL/GB]; 16 Ratho Park Road, Ratho, Midlothian EH28 8NY (GB). **MUIR, Alan, William** [GB/GB]; Howfaulds Farm, Ravenstruther, Lanark ML11 7SL (GB). **REES, David** [GB/SE]; Jacobsdalsgatan 40, S-412 68 Göthenburg (SE).
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- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:
— Without international search report and to be republished upon receipt of that report.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

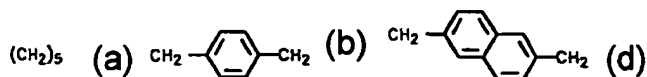
(54) Title: **USE OF CHEMICAL CHELATORS AS REVERSAL AGENTS FOR DRUG-INDUCED NEUROMUSCULAR BLOCK**



(A)



(B)



(57) Abstract: The invention relates to the use of chemical chelators for the preparation of a medicament for the reversal of drug-induced neuromuscular block, to a kit for providing neuromuscular block and its reversal, and to cyclophane derivatives having general formula (A) wherein R is (a), (b) or (c); or general formula (B) wherein X is (a), (b) or (d), or a pharmaceutically acceptable salt thereof.

**Use of Chemical Chelators
as Reversal Agents for Drug-Induced Neuromuscular Block.**

The invention relates to the use of chemical chelators for the preparation of a
5 medicament for the reversal of drug-induced neuromuscular block, and to a
kit for providing neuromuscular block and its reversal.

A neuromuscular blocking agent (NMBA, also called a *muscle relaxant*) is
routinely used during the administration of anaesthesia to facilitate
10 endotracheal intubation and to allow surgical access to body cavities, in
particular the abdomen and thorax, without hindrance from voluntary or reflex
muscle movement. NMBAs are also used in the care of critically-ill patients
undergoing intensive therapy, to facilitate compliance with mechanical
ventilation when sedation and analgesia alone have proved inadequate.

15

Based on their mechanisms of action, NMBAs are divided into two categories:
depolarizing and non-depolarizing. Depolarizing neuromuscular blocking
agents bind to nicotinic acetylcholine receptors (nAChRs) at the
neuromuscular junction in a way similar to that of the endogenous
20 neurotransmitter acetylcholine. They stimulate an initial opening of the ion
channel, producing contractions known as fasciculations. However, since
these drugs are broken down only relatively slowly by cholinesterase
enzymes, compared to the very rapid hydrolysis of acetylcholine by
acetylcholinesterases, they bind for a much longer period than acetylcholine,
25 causing persistent depolarization of the end-plate and hence a neuromuscular
block. Succinylcholine (suxamethonium) is the best known example of a
depolarizing NMBA.

Non-depolarizing neuromuscular blocking agents compete with acetylcholine
for binding to muscle nAChRs, but unlike depolarizing NMBAs, they do not
30 activate the channel. They block the activation of the channel by acetylcholine
and hence prevent cell membrane depolarization, and as a result, the muscle
will become flaccid. Most clinically-used NMBAs belong to the non-

depolarizing category. These include tubocurarine, atracurium, (cis)atracurium, mivacurium, pancuronium, vecuronium, rocuronium and rapacuronium (Org 9487).

5 At the end of surgery or a period of intensive care, a reversal agent of NMBAs is often given to the patient to assist the recovery of muscle function. Most commonly used reversal agents are inhibitors of acetylcholinesterase (AChE), such as neostigmine, edrophonium and pyridostigmine. Because the mechanism of action of these drugs is to increase the level of acetylcholine at
10 the neuromuscular junction by inhibiting the breakdown of acetylcholine, they are not suitable for reversal of depolarizing NMBAs such as succinylcholine. The use of AChE inhibitors as reversal agents leads to problems with selectivity, since neurotransmission to all synapses (both somatic and autonomic) involving the neurotransmitter acetylcholine is potentiated by
15 these agents. This non-selectivity may lead to many side-effects due to the non-selective activation of muscarinic and nicotinic acetylcholine receptors, including bradycardia, hypotension, increased salivation, nausea, vomiting, abdominal cramps, diarrhoea and bronchoconstriction. Therefore in practice, these agents can be used only after or together with the administration of
20 atropine (or glycopyrrolate) to antagonize the muscarinic effects of acetylcholine at the muscarinic receptors in the autonomic parasympathetic neuro-effector junctions (e.g. the heart). The use of a muscarinic acetylcholine receptor (mAChR) antagonist such as atropine causes a number of side-effects, e.g., tachycardia, dry mouth, blurred vision, and furthermore may
25 affect cardiac conduction.

A further problem with anticholinesterase agents is that residual neuromuscular activity must be present (>10 % twitch activity) to allow the rapid recovery of neuromuscular function. Occasionally, either due to hypersensitivity of the patient or accidental overdose, administration of NMBAs can
30 cause complete blockade of neuromuscular function ("deep block"). At present, there is no reliable treatment to reverse such a 'deep block'. Attempts to overcome a 'deep block' with high doses of AChE inhibitors has

the risk of inducing a "cholinergic crisis", resulting in a broad range of symptoms related to enhanced stimulation of nicotinic and muscarinic receptors.

- 5 There is thus a need for an alternative method for reversing the action of NMBAs, i.e. to restore the muscular contractions.

The present invention provides for the use of chemical chelators (or sequestrants) as reversal agents. In one aspect the invention pertains to the use of a chemical chelator capable of forming a guest-host complex for the
10 manufacture of a medicament for the reversal of drug-induced neuromuscular block.

The use of chemical chelators as reversal agents for NMBAs has the advantage that they are effective in reversing the action of both depolarizing and non-depolarizing NMBAs, since chemical chelators do not compete with
15 the NMBA for binding to nAChRs. Their use does not increase the level of acetylcholine and therefore they produce fewer side effects than AChE-based reversal agents. In addition, there is no need for the combined use of a AChE inhibitor and a mAChR antagonist (e.g., atropine). The chemical chelators of
20 the invention may further be safely employed for the reversal of 'deep block'.

The term chemical chelator (or sequestrant), as used in the present invention, means any organic compound which can engage in host-guest complex formation with a neuromuscular blocking agent. The chemical chelator acts as
25 the host molecule, the neuromuscular blocking agent being the guest molecule. The specific molecular complex, the guest-host complex, is defined as an organised chemical entity resulting from the association of two or more components held together by noncovalent intermolecular forces.

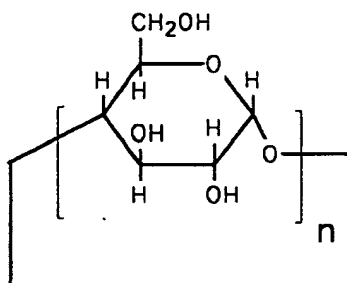
- 30 The chemical chelators (or sequestrants), according to the invention, are host molecules selected from various classes of, mostly cyclic, organic compounds which are known for their ability to form inclusion complexes with various

organic compounds in aqueous solution, e.g. cyclic oligosaccharides, cyclophanes, cyclic peptides, calixarenes, crown ethers and aza crown ethers. Formation of inclusion complexes (also called encapsulation, or chemical chelation) is part of the well-known area of 'supramolecular chemistry' or 'host-guest chemistry'. Many cyclic organic compounds are known to be capable of forming an inclusion complex with another molecule, organic or inorganic. The structures and chemistry of these compounds are well documented (*Comprehensive Supramolecular Chemistry*, Volumes 1-11, Atwood J.L., Davies J.E.D., MacNicol D.D., Vogtle F., eds; Elsevier Science Ltd., Oxford, UK, 1996).

Preferred chemical chelators for use with the present invention are cyclic oligosaccharides, cyclophanes and calixarenes.

Examples of cyclic oligosaccharides suitable for use with the invention are the cyclodextrins, a category of naturally occurring cyclomaltooligosaccharides, the cyclomannins (5 or more α -D-mannopyranose units linked at the 1,4 positions by α linkages), the cyclogalactins (5 or more β -D-galactopyranose units linked at the 1,4 positions by β linkages), the cycloaltrins (5 or more α -D-altropyranose units linked at the 1,4 positions by α linkages), each of which are capable of forming guest-host complexes. Cyclic oligosaccharides of different monosaccharide compositions, accessible through total chemical synthesis, represent further chemical chelators capable of interaction with a neuromuscular blocking agent. For example, cyclo-[(1-4)- α -L-rhamno-pyranosyl-(1-4)- α -D-mannopyranosyl]tetraoside, was found to be effective in reversal of the action of the neuromuscular blocking agent rocuronium bromide.

A particularly preferred class of cyclic oligosaccharide chelators according to the invention is formed by the cyclodextrins:



$n = 6 - 9$

Cyclodextrins are cyclic molecules containing six or more α -D-glucopyranose units linked at the 1,4 positions by α linkages as in amylose. As a consequence of this cyclic arrangement, the cyclodextrins exist as conical shaped molecules with a lipophilic cavity which can attract guest molecules whilst the outside is more hydrophilic and water-soluble. Cyclodextrins composed of six, seven, eight and nine glucopyranose units are commonly known as α -, β -, γ - and δ -cyclodextrins, respectively.

Both the native cyclodextrins (α , β , γ) which are prepared by enzymatic degradation of starch, and especially a number of chemically modified forms thereof, have already found, by virtue of their ability to form guest-host complexes, numerous applications, especially in the pharmaceutical field. Stella and Rajewski (Pharmaceutical Research, 14, 556-567, 1997) have recently reviewed pharmaceutical applications of the cyclodextrins. The major applications are in the pharmaceutical formulations of drugs in order to solubilize and/or to stabilize a drug for oral, nasal, ophthalmic, dermal, rectal and parenteral administration.

The term cyclodextrin as used in relation to the present invention includes both the native cyclodextrins and chemically modified forms thereof.

An overview on such chemically modified cyclodextrins as drug carriers in drug delivery systems is described by Uekama et al. (Chemical Reviews 1998, 98, 2045-2076). Chemical modification of cyclodextrins can be made directly on the native α -, β - or γ -cyclodextrin rings by reacting a chemical reagent (nucleophiles or electrophiles) with a properly functionalised cyclo-

dextrin (for an recent overview of methods for the selective modification of cyclodextrins see Khan A.R. et al. *Chem. Rev.* 1998, 98, 1977-1996). To date, more than 1,500 cyclodextrin derivatives have been made by chemical modification of native cyclodextrins (Jicsinszky L. et al *Comprehensive*
5 *Supramolecular Chemistry*, Volume 3. Cyclodextrins, Atwood J.L., Davies J.E.D, MacNicol D.D., Vogtle F., eds; Elsevier Science Ltd., Oxford, UK, 1996, pp 57-188).

Many direct modifications of a native cyclodextrin result in a mixture of
10 isomers without precisely defined positions of substitution. Such a mixture of positional isomers is often referred to as a statistic mixture, the number of substituents attached at each cyclodextrin molecule in such a statistic mixture being expressed as the average degree of substitution (DS). Most cyclodextrin derivatives studied for pharmaceutical applications are statistic
15 mixtures (Szente L. and Szejtli J., *Adv. Drug Delivery Rev.* 1999, 36, 17-28). Direct modification of a cyclodextrin does not alter the constitution or the configuration of the repeating α -D-glucopyranosyl units.

Cyclodextrins can also be prepared by *de novo* synthesis, starting with
20 glucopyranose (Gattuso G. et al *Chem. Rev.* 1998, 98, 1919-1958). In this way, one can prepare not only the naturally occurring cyclic (1 \rightarrow 4)-linked cyclodextrins but also the cyclic (1 \rightarrow 3)-, (1 \rightarrow 2)-, and (1 \rightarrow 6)-linked oligopyranosides. Such a synthesis can be accomplished by using various chemical reagents or biological enzymes such as cyclodextrin transglycosylase. By using different sugar units as the starting materials, one can thereby
25 prepare various homogeneous or heterogeneous cyclic oligosaccharides.

Chemical modification of cyclodextrins is thus known to modulate their properties and can be used for the design of reversal agents selective for a specific neuromuscular blocking agent.

30 It will be clear to the skilled person that for a particular neuromuscular blocking agent a chemical chelator host can be developed having a hydrophobic cavity of a shape and size adapted to the guest molecule, while

in addition to the hydrophobic interactions between the host and the guest charge interactions can be of importance for complex formation. Since the chemical chelators of the invention are for parenteral application they will have to be water-soluble. A specific host molecule can be designed and prepared to contain functionalities complementary to those of the guest molecule in such a manner that it results in maximum intermolecular interaction via for example hydrogen-bond, hydrophobic, electrostatic, van der Waals, and π - π interactions. Thus, for example, for a guest molecule containing basic functional groups or positive charge, a host molecule containing acidic functional groups or negative charge could be made to increase ionic interaction between the guest and the host. When such a host-guest complex is formed via inclusion or partial inclusion, the cavity size of the host molecule is also very important

The interaction between a chemical chelator and a neuromuscular blocking agent can be analyzed by physical methods such as nuclear magnetic resonance spectroscopy (nmr) and microcalorimetry.

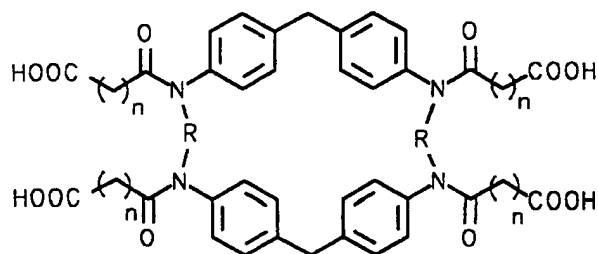
The most preferred cyclodextrins for use in the invention are γ -cyclodextrin and derivatives thereof.

Many of the commonly used neuromuscular blocking agents, or muscle relaxants, such as rocuronium, pancuronium, vecuronium, mivacurium, atracurium, (cis)atracurium, succinylcholine and tubocurarine, are compounds having 1 or 2 cationic sites when in neutral aqueous medium. Cyclodextrins having anionic sites in their structure are among the preferred chemical chelators according to the invention.

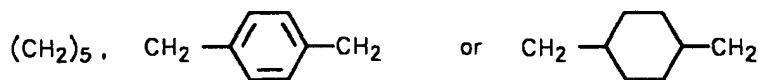
The preference for anionic chemical chelators for the reversal of the above mentioned neuromuscular blocking agents also applies for chemical chelators of the invention which belong to the cyclophanes.

Cyclophanes are a class of bridged aromatic compounds which have well-defined hydrophobic inclusion cavities constructed by aromatic rings incorporated in their macrocyclic structures. By introducing polar and hydrophilic functional groups such as hydroxyls and carboxyls into the artificial host compounds, cyclophanes can be made water-soluble and suitable for forming inclusion complex in aqueous media (Vogtle F. et al. *Comprehensive Supramolecular Chemistry*, Volume 2. Molecular recognition: Receptors for molecular guests, Atwood, J.L., Davies, J.E.D., MacNicol, D.D., Vogtle, F., eds; Elsevier Science Ltd., Oxford, UK, 1996, pp 211-266). Water soluble anionic cyclophanes are described by Miyake et al. (Tetr. Letters 32, 7295-7298, 1991; Chem. Pharm. Bull. 41, 1211-1213, 1993) as hosts for cationic aromatic guests. Analogously, cationic cyclophanes were shown to form inclusion complexes in aqueous solution with anionic and neutral aromatic compounds.

In a preferred embodiment of the invention the chemical chelator is chosen from cyclophanes having the general formula A

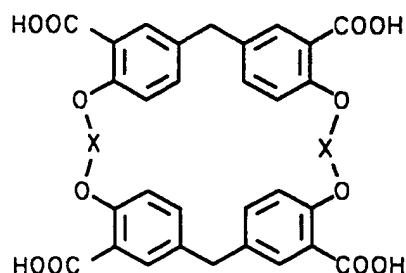


wherein R is

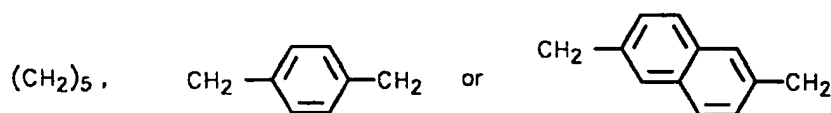


and n is 1-5; or the general formula B

9



wherein X is



- 5 The compound according to formula A, wherein R is CH_2 -phenyl- CH_2 and n is 2, i.e. N, N', N'', N'''-tetrakis (3-carboxypropionyl)-3,4,5,6,7,8,26,27,28,-29,30,31-dodecahydro-1,10,24,33-tetraaza[2.2.1.2.2.1]paracyclophane (compound **23**), and the compound according to formula B wherein X is CH_2 -phenyl- CH_2 , i.e. 1,10,24,33-tetraoxa-12,20,35,43-tetracarboxy-[2.2.1.2.2.1]-
- 10 paracyclophane (compound **18**) is a preferred cyclophane derivative to reverse the action of rocuronium bromide.

In a further aspect the invention provides for novel paracyclophane derivatives having formula A or Formula B, as defined above, or

15 pharmaceutically acceptable salts thereof. Examples of such salts are the potassium, sodium and ammonium salts and the like.

The chemical chelators for use in the invention are administered parenterally. The injection route can be intravenous, subcutaneous, intradermal,

20 intramuscular, or intra-arterial. The intravenous route is the preferred one. The exact dose to be used will necessarily be dependent upon the needs of the individual subject to whom the medicament is being administered, the degree of muscular activity to be restored and the judgement of the anaesthetist/critical-care specialist. Extracorporeal application of the chemical

chelators of the invention, for instance by mixing of the chemical chelator with the blood during dialysis or during plasmapheresis, is also contemplated.

In a further aspect the invention relates to a kit for providing neuromuscular block and its reversal comprising (a) a neuromuscular blocking agent, and (b) a chemical chelator capable of forming a guest-host complex with the neuromuscular blocking agent. With a kit according to the invention is meant a formulation, which contains separate pharmaceutical preparations, i.e. the neuromuscular blocking agent and a chemical chelator, i.e. the reversal agent. The components of such a kit of parts are to be used sequentially, i.e. the neuromuscular blocking agent is administered to a subject in need thereof, which is followed, at a point in time when restoration of muscle function is required, by the administration of the reversal agent, i.e. a chemical chelator capable of forming a guest-host complex with the neuromuscular blocking agent.

A preferred kit, according to the invention, contains a chemical chelator selected from the group consisting of a cyclic oligosaccharide and a cyclophane, and a neuromuscular blocking agent which is selected from the group consisting of rocuronium, vecuronium, pancuronium, rapacuronium, mivacurium, atracurium, (cis)atracurium, tubocurarine and suxamethonium. A particularly preferred kit of the invention comprises rocuronium, as the neuromuscular blocking agent, and γ -cyclodextrin, or a derivative thereof, as the chemical chelator.

Mixed with pharmaceutically suitable auxiliaries and pharmaceutically suitable liquids, e.g. as described in the standard reference, Gennaro et al., Remington's Pharmaceutical Sciences, (18th ed., Mack Publishing Company, 1990, Part 8: Pharmaceutical Preparations and Their Manufacture; see especially Chapter 84 on "Parenteral preparations, pp. 1545-1569; and Chapter 85 on "Intravenous admixtures", pp. 1570-1580) the chemical

chelators can be applied in the form of a solution, e.g. for use as an injection preparation.

Alternatively, the pharmaceutical composition may be presented in unit-dose or multi-dose containers, for example sealed vials and ampoules, and may be
5 stored in a freeze dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example, water prior to use.

The invention further includes a pharmaceutical formulation, as hereinbefore described, in combination with packaging material suitable for said composition, said packaging material including instructions for the use of the
10 composition for the use as hereinbefore described.

The invention is illustrated in the following examples.

Example 1.

5

Cyclodextrin derivatives.

Some of the cyclodextrin derivatives that were employed to demonstrate their activity as reversal agents according to the invention were commercially available:

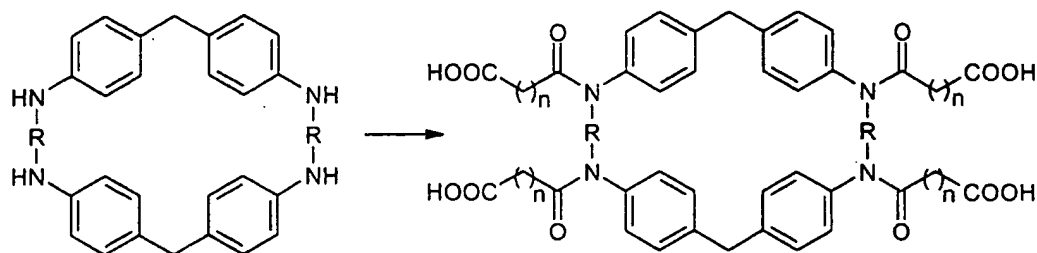
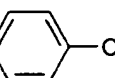
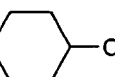
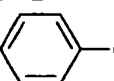

Commercial Source

Compound <u>1</u> : α -cyclodextrin (α -CD)	Wacker-Chemie GmbH, Munich, Germany; or ALDRICH
Compound <u>2</u> : carboxymethyl- β -CD (DS= 3.5) sodium salt	Wacker-Chemie GmbH, Munich, Germany
Compound <u>3</u> : 2-hydroxy-3-trimethylammonio propyl-- β -CD (DS= 3.5)	Wacker-Chemie GmbH, Munich, Germany
Compound <u>4</u> : per 2,6-dimethyl-- β -CD (DS=12.6)	Wacker-Chemie GmbH, Munich, Germany
Compound <u>5</u> : β -cyclodextrin-phosphate sodium salt (DS=3)	CycloLab, Ltd. Budapest, Hungary
Compound <u>6</u> : β -cyclodextrin-phosphate sodium salt (DS=8)	CycloLab, Ltd. Budapest, Hungary
Compound <u>7</u> : carboxymethyl- β -CD (DS= 3-3.5)	CycloLab, Ltd. Budapest, Hungary
Compound <u>8</u> : carboxyethyl- β -CD (DS= 3)	CycloLab, Ltd. Budapest, Hungary
Compound <u>9</u> : β -cyclodextrin (β -CD)	Wacker-Chemie GmbH, Munich, Germany; or ALDRICH
Compound <u>10</u> : 2-hydroxypropyl- β -CD	RBI, Natick, MA 01760, USA
Compound <u>11</u> : γ -cyclodextrin-phosphate sodium salt (DS=3)	CycloLab, Ltd. Budapest, Hungary
Compound <u>12</u> : γ -cyclodextrin-phosphate sodium salt (DS=7)	CycloLab, Ltd. Budapest, Hungary
Compound <u>13</u> : carboxymethyl- γ -CD (DS=3.2)	CycloLab, Ltd. Budapest, Hungary
Compound <u>14</u> : carboxyethyl- γ -CD (DS= 3.8)	CycloLab, Ltd. Budapest, Hungary
Compound <u>15</u> : γ -cyclodextrin (γ -CD)	Wacker-Chemie GmbH, Munich, Germany; or FLUKA
Compound <u>16</u> : 2-hydroxypropyl- γ -CD (DS= 4)	RBI, Natick, MA 01760, USA

- 10 - DS means the degree of substitution, which is the mean number of hydroxy functions which carry the pertinent substituent.
- compounds 2 and 7 are the same, be it from different suppliers.

Example 2.**Cyclophane derivatives.**

Nomenclature: the term *paracyclophane* refers to a family of compounds in which one or more benzene rings are built into a carbocyclic ring system and in which the *p*-positions of the benzene rings are part of the ring system. The conventional numbering used below for *paracyclophane* ring systems is that described by Cram and Abell (J.Am.Chem.Soc. 1955, 77, 1179-1186) _

10 **A: Tetraaza-paracyclophanes derivatives:**I: R = (CH₂)₅II: R = CH₂--CH₂III: R = CH₂--CH₂Compound 19: R = (CH₂)₅, n = 1Compound 20: R = (CH₂)₅, n = 3Compound 21: R = (CH₂)₅, n = 2Compound 22: R = CH₂--CH₂ n = 2Compound 23: R = CH₂--CH₂ n = 2**Scheme A**

15

Scheme A depicts the structures of the N-(carboxy)acylated cyclophane derivatives **19-23**, which were prepared by acylation of the parent cyclophanes (see Soga T. et al *Tetrahedron Lett.* 1980, 4351-4, for synthesis thereof) (1,7,21,27-tetraaza[7.1.7.1]paracyclophane (I), 1,10,24,33-tetraaza-
20 [2.2.1.2.2.1]paracyclophane (II) and 3,4,5,6,7,8,26,27,28,29,30,31-dodeca-

hydro-1,10,24,33-tetraaza[2.2.1.2.2.1]paracyclophane (III) with the appropriate activated acid derivative.

A1: Compound 21:

- 5 N, N', N'', N'''-Tetrakis(3-carboxypropionyl)-1, 7, 21, 27-tetraaza[7.1.7.1]para - cyclophane.

To a suspension of 1,7,21,27-tetraaza[7.1.7.1]paracyclophane (400 mg, 0.75 mmol) in dichloromethane (5 ml) was added triethylamine (1.05 ml, 7.52 mmol) followed by 3-(methoxycarbonyl)propionyl chloride (0.93 ml, 7.52 mmol) dissolved in dichloromethane (3 ml). The reaction was stirred under an atmosphere of nitrogen for 12 h. The reaction was diluted with dichloromethane (20 ml) and washed with water (2 x 20 ml), dried (MgSO₄) and the solvent removed *in vacuo* to give a yellow oil, which was purified by chromatography on silica gel eluting with 5% methanol in dichloromethane.

15 The resultant product crystallised on standing. The product was recrystallised from chloroform/ether to give N, N', N'', N'''-tetrakis[3-(methoxycarbonyl)propionyl]-1, 7, 21, 27-tetraaza[7.1.7.1]para cyclophane (480 mg, 0.48 mmol, 65%). MS (EI) m/z 989 (M+H)⁺, ¹H NMR (CDCl₃) δ 1.27 (m, 4H), 1.45 (m, 5H), 1.64 (m, 3H), 2.25 (t, J 6.8, 8H), 2.56 (t, J 6.8, 8H), 3.61 (t, J 7.6, 8H), 3.65 (s, 12H), 4.02 (s, 4H), 7.09 (d, J 8.1, 8H), 7.20 (d, J 8.1, 8H); ¹³C NMR (CDCl₃) δ 24.45, 27.85, 29.17, 29.37, 40.73, 49.47, 51.68, 128.47, 130.27, 140.15, 140.60, 171.35, 173.84.

A mixture of the above tetramethylester (440 mg, 0.45 mmol), potassium hydroxide pellets (2.51 g, 45 mmol), methanol (9 ml) and water (25 ml) was heated to reflux for 4 h. The reaction was cooled to room temperature, most of the solvent removed *in vacuo* and the residue acidified with 2N HCl. The resultant precipitate was filtered and dried then recrystallised from MeOH/H₂O to give the title compound **21** (142 mg, 0.15 mmol, 34%).

MS (EI) m/z 931 (M-H)⁻, ¹H NMR (CD₃OD) δ 1.28 (m, 4H), 1.44 (m, 8H), 2.25 (m, 8H), 2.49 (m, 8H), 3.60 (m, 8H), 4.05 (s, 4H), 7.16 (d, J 7.72, 8H), 7.30 (d, J 7.72, 8H), ¹³C NMR (CDCl₃) δ 23.95, 27.44, 28.94, 29.11, 39.86, 48.62, 128.21, 129.79, 140.01, 140.35, 170.01, 173.07, I.R. (KBr) 1736, 1656 cm⁻¹.

In a similar manner were prepared:

A2: N, N', N'', N'''-Tetrakis(carboxyacetyl)-1, 7, 21, 27-tetraaza[7.1.7.1] paracyclophane (**Compound 19**) starting from 1, 7, 21, 27-tetraaza[7.1.7.1]-paracyclophane and methyl malonyl chloride.

MS (EI) m/z 877 (M+H)⁺, ¹H NMR (DMSO) δ 1.21 (m, 4H), 1.36 (m, 8H), 2.97 (m, 8H), 3.53 (m, 8H), 3.99 (s, 4H), 7.15 (d, J 7.95, 8H), 7.27 (d, J 7.95, 8H), 12.40 (s, 4H), ¹³C NMR (DMSO) δ 23.79, 27.19, 39.39, 41.44, 48.59, 128.04, 129.83, 145.05, 141.02, 165.69, 169.23, I.R. (KBr) 1736, 1625 cm⁻¹.

10

A3: N, N', N'', N'''-Tetrakis(4-carboxybutyryl)-1, 7, 21, 27-tetraaza[7.1.7.1] paracyclophane (**Compound 20**) starting from 1,7,21,27-tetraaza[7.1.7.1]-paracyclophane and methyl 4-(chloroformyl)butyrate.

MS (EI) m/z 989 (M+H)⁺, ¹H NMR (CD₃OD) δ 1.28 (m, 4H), 1.44 (m, 8H), 1.76 (m, 8H), 2.07 (t, J 7.5, 8H), 2.19 (t, J 7.5, 8H), 3.62 (m, 8H), 4.05 (s, 4H), 7.11 (d, J 8.17, 8H), 7.27 (d, J 8.17, 8H), ¹³C NMR (CDCl₃) δ 21.86, 25.54, 28.72, 34.02, 34.41, 41.70, 50.60, 129.50, 131.47, 141.82, 142.55, 174.69, 177.69, I.R. (KBr) 1736, 1656 cm⁻¹.

20 **A4:** N, N', N'', N'''-Tetrakis(3-carboxypropionyl)1,10,24,33-tetraaza-[2.2.1.2.2.1]paracyclophane (**Compound 22**) starting from cyclophane II and 3-(methoxycarbonyl)propionyl chloride.

¹H NMR (DMSO) δ 2.22 (m, 8H), 2.42 (m, 8H), 3.93 (s, 4H), 4.75 (s, 8H), 6.96 (d, J 8.27, 8H), 7.00 (s, 8H), 7.16 (d, J 8.27, 8H), 11.90 (bs, 1H), ¹³C NMR (DMSO) δ 28.83, 29.10, 39.93, 51.51, 125.32, 127.83, 128.22, 129.43, 136.36, 139.88, 14.00, 170.69, 173.52, I.R. (KBr) 1727, 1650 cm⁻¹.

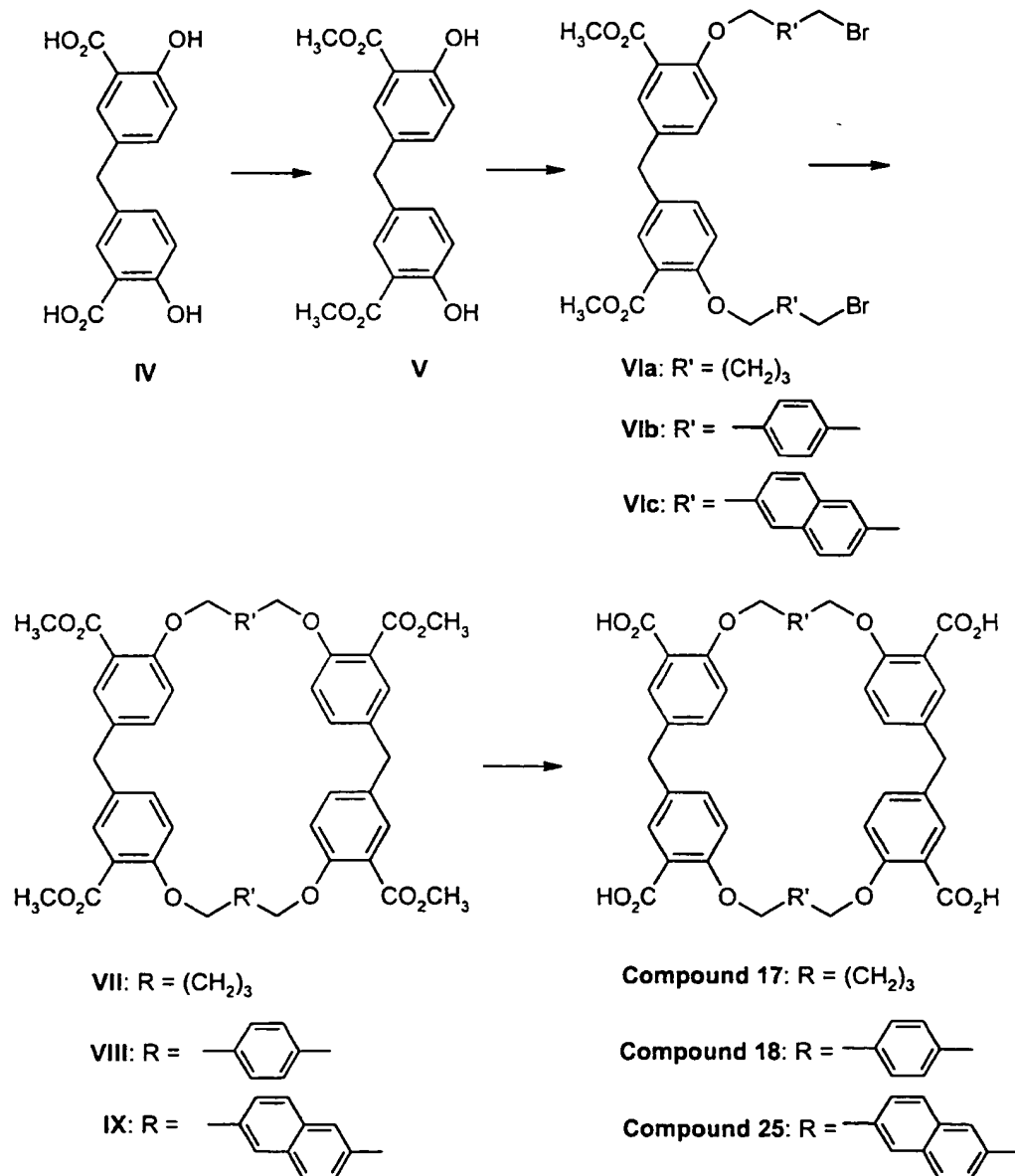
25 **A5:** N, N', N'', N'''-Tetra (3-carboxypropionyl)-3,4,5,6,7,8,26,27,28,29,30,31-dodecahydro-1,10,24,33-tetraaza[2.2.1.2.2.1]paracyclophane (**Compound 23**) starting from cyclophane III and 3-(methoxycarbonyl)propionyl chloride.

MS (EI) m/z 1012 (M-H)⁻, ¹H NMR (DMSO) δ 0.76 (m, 8H), 1.51 (m, 4H), 1.67 (m, 8H), 2.15 (m, 8H), 2.36 (t, J 6.60, 8H), 3.37 (s, 8H), 3.97 (s, 4H), 7.19 (d,

J 8.08, 8H), 7.30 (d, J 8.08, 8H), 11.86 (bs, 4H), ^{13}C NMR (DMSO) δ 28.97, 29.09, 36.13, 52.33, 55.59, 128.09, 129.72, 141.55, 170.65, 173.52, I.R. (KBr) 1727, 1652 cm^{-1}

5 **B.** Tetraoxa-paracyclophane derivatives.

The ether-linked cyclophanes (compounds **17**, **18** and **25**) can be synthesised by a ring construction as shown in **Scheme B**.



Scheme B

The commercially available 5,5'-methylenedisalicylic acid **IV** was protected as methyl ester **V** which was then alkylated with an appropriate dihalide to yield **VI**. Reaction of dihalide **VI** with an equivalent diphenol **IV** gave the cyclophanes **VII-IX** which gave the desired carboxylic acid derivatives upon saponification.

B1: Compound **17**: 1,7,21,27-tetraoxa-9,17,29,37-tetracarboxy[7.1.7.1]paracyclophane.

10 3,3'-Dimethoxycarbonyl-4,4'-dihydroxydiphenylmethane (**V**)

To methanol (100 ml) saturated with hydrogen chloride gas was added 3,3'-dicarboxy-4,4'-dihydroxydiphenylmethane (10 g, 34.69 mmol) portion-wise over 30 min. The mixture was then heated to reflux for 3 h, cooled to room temperature and re-saturated with hydrogen chloride gas. After a further 15 8 h heating at reflux the solvent was removed *in vacuo* and the product purified by chromatography on silica gel eluting with 25% ethyl acetate/petroleum ether to give the title compound (2.40 g, 7.59 mmol, 22%). ¹H NMR (CDCl₃) δ 3.84 (s, 2H), 3.92 (s, 6H), 6.90 (d, J 8.0, 2H), 7.25 (dd, J 8.0, 1.0, 2H), 7.63 (d, J 1.0, 2H), 10.65 (s, 2H).

20

4,4'-Bis(5-bromopentoxymethyl)-3,3'-dicarboxymethyl-4,4'-dihydroxydiphenylmethane (**Vla**)

To a stirred suspension of 1,4-dibromopentane (21.8 g, 94.9 mmol) and K₂CO₃ (13.1 g, 94.9 mmol) in dry dimethylformamide (380 ml) at 60 °C, 25 under an atmosphere of nitrogen, was added dropwise a solution of 3,3'-dicarboxymethyl-4,4'-dihydroxydiphenylmethane (3.0 g, 9.49 mmol) in dry dimethylformamide (190 ml). The resultant mixture was heated a further 1h, cooled and filtered. The dimethylformamide was removed *in vacuo* and the product purified by chromatography eluting with 1% methanol in dichloromethane followed by a second purification eluting with 10% ethyl acetate/- 30 heptane to give the title compound (3.51 g, 5.73 mmol, 60%).

¹H NMR (CDCl₃) δ 1.60-2.02 (m, 12H), 3.44 (t, J 7.0, 4H), 3.87 (s, 8H), 4.01 (t, J 6.5, 4H), 6.87 (d, J 8.0, 2H), 7.22 (dd, J 8.0, 1.0, 2H), 7.60 (d, J 1.0, 2H).

1,7,21,27-tetraoxa-9,17,29,37-tetra(methoxycarbonyl)[7.1.7.1]paracyclophane
(VII)

A solution of 4,4'-bis(5-bromopentoxy)-3,3'-dicarboxymethyl-4,4'-dihydroxydiphenylmethane (3.51 g, 5.74 mmol) and 3,3'-dicarboxymethyl-4,4'-dihydroxydiphenylmethane (1.81 g, 5.74 mmol) in dry dimethylformamide (230 ml) was added dropwise *via* syringe pump to a stirred suspension of K₂CO₃ (7.92 g, 57.4 mmol) in dry dimethylformamide (340 ml) at 80 °C over a period of 3 h. After stirring a further 4.5 h at 80 °C and 12 h at room temperature the reaction mixture was filtered and the dimethylformamide removed *in vacuo*. The product was purified by chromatography on silica gel eluting with 1% methanol/dichloromethane to give the title compound (0.47 g, 0.6 mmol, 10.5%).

NMR (CDCl₃) δ 1.58-1.95 (m, 12H), 3.82 (s, 16H), 4.05 (t, J 8.0, 8H), 6.82 (d, J 8.0, 4H), 7.16 (dd, J 8.0, 1.0, 4H), 7.58 (d, J 1.0, 4H).

1,7,21,27-tetraoxa-9,17,29,37-tetracarboxy[7.1.7.1]paracyclophane (**17**)

To a suspension of 1,7,21,27-tetraoxa-9,17,29,37-tetra(methoxycarbonyl)[7.1.7.1]paracyclophane (0.47 g, 0.612 mmol) in methanol-water (3:1, 40 ml) was added solid sodium hydroxide (0.49 g (12.2 mmol)). The resultant mixture was heated to reflux for 1h, then tetrahydrofuran (5 ml) was added and the mixture again heated to reflux for 2 h. The solvent volume was reduced by half *in vacuo* and insoluble material removed by filtration. The filtrate was acidified with conc hydrochloric acid and the resultant precipitate filtered dried, and further washed with methanol-water before drying to give the title compound (160 mg, 0.22 mmol, 45%).

MS (EI) m/z 711 (M-H)⁻, ¹H NMR (DMSO) δ 1.51 (m, 4H), 1.68 (m, 8H), 3.81 (s, 4H), 3.99 (t, J 5.6, 8H), 6.96 (m, 4H), 7.20 (m, 4H), 7.43 (m, 4H), 12.25 (bs, 4H), ¹³C NMR (DMSO) δ 21.57, 27.81, 38.72, 68.47, 113.99, 121.77, 130.28, 132.60, 133.21, 155.61, 167.27, I.R. (KBr) 1743 cm⁻¹.

In a similar manner were prepared

5 **B2:** 1,10,24,33-tetraoxa-12,20,35,43-tetracarboxy-[2.2.1.2.2.1]paracyclophane, compound **18**, starting from 3,3'-dicarboxymethyl-4,4'-dihydroxydiphenyl methane and α,α' -dibromo-*p*-xylene.

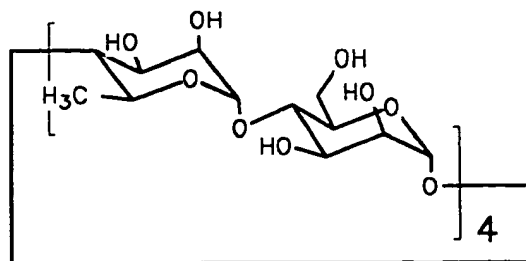
MS (EI) m/z 779 (M-H)⁻, ¹H NMR (DMSO) δ 3.75 (s, 4H), 5.15 (s, 8H), 6.91 (d, J 8.58, 4H), 7.20 (m, 4H), 7.32 (m, 8H), 7.49 (m, 4H), 12.40 (bs, 4H), ¹³C
10 NMR (DMSO) δ 68.69, 84.57, 114.52, 126.54, 129.98, 132.32, 133.81, 136.28, 154.60, 167.50, I.R. (KBr) 1733 cm⁻¹.

B3: Compound **25** starting from 3,3'-dicarboxymethyl-4,4'-dihydroxydiphenyl methane and 2, 6-(dibromomethyl)naphthalene (Golden, J. H., J. Chem. Soc.
15 1961, 3741).

MS (EI) m/z 879 (M-H)⁻, ¹H NMR (DMSO) δ 3.76 (s, 4H), 5.30 (s, 8H), 6.90 (m, 4H), 7.19 (m, 4H), 7.49 (m, 8H), 7.74 (m, 4H), 7.83 (m, 4H), 12.60 (bs, 4H), ¹³C NMR (DMSO) δ 69.21, 83.84, 114.73, 121.88, 125.25, 125.37, 127.96, 130.34, 132.04, 132.67, 133.97, 135.03, 154.91, 167.52, I.R. (KBr)
20 1731 cm⁻¹.

Example 3.

Compound **24**: cyclo[(1-4)- α -L-rhamnopyranosyl-(1-4)- α -D-mannopyranosyl]-tetraoside.



25

The synthesis of this cyclic octasaccharide is described by Ashton et al. in Chem. Eur. J. 1996, 2, 580-591.

Example 4

Complexation of Rocuronium bromide by chemical chelators.

All the ^1H spectra (303 K) were recorded at 400.13 MHz with 128 scans, $\text{sw} =$
5 12 ppm, $\text{TD} = 32$ k and zero filled to 64 k real points in processing. All experiments were measured at 303K.

Determination of stoichiometry

Stock solutions of rocuronium bromide and γ -cyclodextrin (**15**) were prepared,
10 both with a concentration of 6.02 mM. From these, sixteen solutions were prepared (with the mole % of rocuronium ranging from 0-100) by taking aliquots of 0-800 μl of the rocuronium bromide solution. Aliquots of the γ -cyclodextrin solution, 800-0 μl were added to make the solution a total volume of 800 μl and 100 mole % (i.e. 6.02 mM of [rocuronium bromide +
15 γ -cyclodextrin]). ^1H -NMR spectra were recorded as described above.

If the chemical shift change of H_{α} in rocuronium bromide is defined as $\Delta\delta$ then a plot of $[\Delta\delta^*(\text{mole \% rocuronium bromide})]$ vs. [mole % rocuronium bromide] gives a so called Job Plot by the method of continuous variation (Connors K.A.: *Binding constants, The measurement of Molecular Complex*
20 *Stability*; Wiley-Interscience; New York, 1987, pp 24-28). The maximum in this plot is indicative for the stoichiometry of the complex. The Job Plot for the rocuronium bromide/ γ -cyclodextrin complex has a maximum at 50 mole % rocuronium, indicating that rocuronium bromide and γ -cyclodextrin form a 1:1 complex.

25

Determination of the association constants.

A stock solution of rocuronium bromide: 0.821 mM in D_2O was prepared. Stock solutions of β -cyclodextrin (**9**) and γ -cyclodextrin (**15**), both with concentrations of 13.1, 6.57, 1.64, and 0.411 mM in D_2O , were prepared.
30 Aliquots of 50-400 μl of these solutions were then removed and made up to 400 μl with D_2O (where required) and mixed with 400 μl of the rocuronium bromide solution. To extend the data range for γ -cyclodextrin (**15**)

experiments to higher cyclodextrin concentrations three additional solutions were prepared: 16.4, 24.6, 32.8 mM in 400 μ l D₂O. As before, these solutions were mixed with 400 μ l of the rocuronium bromide solution.

¹H NMR spectra were recorded as described above.

- 5 The association constants of the complexes were derived from the plots of proton chemical shift changes of the cyclodextrin and/or rocuronium bromide signals ($\Delta\delta$) versus the mole % cyclodextrin, using a curve fitting method (Loukas Y.L., J. Pharm. Pharmacol. 1997, 49, 941; Bisson A.P., et al. Chem. Eur. J. 1998, 4, 845). The association constants are listed in Table are listed
10 in Table A.

Table A. Association constants of 1:1 complex of rocuronium bromide and cyclic host compounds (K_a , M⁻¹), determined by NMR spectroscopy at 303 K.

Chemical chelator	Association constant (K_a , M ⁻¹)
β -cyclodextrin (<u>9</u>)	3,100 – 3,900*
γ -cyclodextrin (<u>15</u>)	10,000 – 20,400#

- 15 *protons of CH₃-19 [rocuronium] and H₃ [β -cyclodextrin (9)] measured.

#protons of H_{9 α} [rocuronium] and H_{3,5} [γ -cyclodextrin (15)] measured.

20 **Example 5.**

Reversal of neuromuscular blockade *in vivo*:

Anaesthetized guinea pig.

- 25 Male Dunkin-Hartley guinea pigs (bodyweight: 600-900 g) were anaesthetized by i.p. administration of 10 mg/kg pentobarbitone and 1000 mg/kg urethane. After tracheotomy, the animals were artificially ventilated using a Harvard small animal ventilator. A catheter was placed into the carotid artery for

continuous monitoring of arterial blood pressure and the taking of blood samples for blood gas analysis. Heart rate was derived from the blood pressure signal. The sciatic nerve was stimulated (rectangular pulses of 0.5 ms duration at 10 s (0.1 Hz) intervals at a supramaximal voltage, using a Grass S88 Stimulator) and the force of M. gastrocnemius contractions was measured using a Grass FT03 force-displacement transducer. Contractions, blood pressure and heart rate were recorded on a multichannel Grass 7D recorder. Catheters were placed in both jugular veins. One catheter was used for the continuous infusion of a neuromuscular blocking agent. The infusion rate of the neuromuscular blocking agent was increased until a steady-state block of 85-90% was obtained. The other catheter was used for administration of increasing doses of the reversal agent. During continuous infusion of the neuromuscular blocking agent, single doses of increasing concentration of reversal agent were given. At the end of the experiment, the measured force of muscle contractions was plotted against the concentration of reversal agent, and using regression analysis techniques, the 50% reversal concentration was calculated.

Results for the reversal of the neuromuscular block, induced by the muscle relaxants rocuronium bromide (Roc), vecuronium bromide (Vec), pancuronium bromide (Pan), mivacurium chloride (Miv), atracurium besilate (Atr), cis-atracurium (Cis-Atr), tubocurarine chloride (T-C), suxamethonium chloride (Sux; succinylcholine) and rapacuronium bromide (Rap; Org 9487), by means of a series of α -, β - and γ -cyclodextrins (compounds 1 - 16) and modified cyclodextrins are presented in **Table I**. The results demonstrate that the action of each of the neuromuscular blocking agents can be reversed by intravenous administration of a cyclodextrin derivative.

TABLE I Dose (ED_{50} , $\mu\text{mol.kg}^{-1}$) producing 50% reversal of steady-state neuromuscular block in anaesthetized guinea pigs.

Compound*	Roc	Vec	Pan	Miv	Atr	Cis-Atr	T-C	Sux	Rap
1: α -cyclodextrin (α -CD)	1575 (2)	2610 (1)							
2: carboxymethyl- β -CD (DS= 3.5) sodium salt	134 (2)	800 (1)							
3: 2-hydroxy-3-trimethylammonio propyl- β -CD (DS= 3.5)	518 (2)	1400 (1)							
4: per 2,6-dimethyl- β -CD (DS=12.6)	70 (2)	1313 (4)							
5: β -cyclodextrin-phosphate sodium salt (DS=3)	280 (3)								
6: β -cyclodextrin-phosphate sodium salt (DS=8)	120 (2)								
7: carboxymethyl- β -CD (DS= 3-3.5)	139 (3)								
8: carboxyethyl- β -CD (DS= 3)	42 (3)	103 (2)	479 (2)					408 (2)	412 (2)
9: β -cyclodextrin (β -CD)	20 (3)	636 (1)	1050 (2)						358 (3)
10: 2-hydroxypropyl- β -CD	33 (3)	1598 (4)							
11: γ -cyclodextrin-phosphate sodium salt (DS=3)	64 (3)	67 (3)	197 (2)	292 (2)	113 (2)	204 (2)	160 (2)	160 (2)	80 (3)
12: γ -cyclodextrin-phosphate sodium salt (DS=7)	52 (2)								
13: carboxymethyl- γ -CD (DS=3.2)	25 (4)	30 (3)	641 (4)	964 (3)	990 (2)	893 (2)	431 (2)	999 (2)	227 (2)
14: carboxyethyl- γ -CD (DS= 3.8)	7 (3)	25 (3)	141 (3)	399 (2)	421 (2)	1558 (3)	294 (2)	1294 (2)	43 (3)
15: γ -cyclodextrin (γ -CD)	4 (3)	75 (4)	186 (4)	1690 (2)	2793 (4)	1710 (2)	630 (2)	1189 (2)	57 (3)
16: 2-hydroxypropyl- γ -CD (DS= 4)	12 (3)	123 (3)	440 (3)	1674 (2)	828 (2)	878 (2)	909 (2)	1340 (2)	134 (3)

ED_{50} values are the mean of a number of experiments; the number is shown in parenthesis.

Example 6.**Reversal of neuromuscular block *in vitro* :****Isolated mouse hemidiaphragm preparation.**

Hemidiaphragms, with phrenic nerves attached, were removed from euthanized male mice (Institute of Cancer Research; bodyweight: 20-60 g).

The preparations were mounted on a tissue holder and placed in a tissue bath filled with a modified Krebs-Henseleit solution (composition: 118 mM NaCl, 30 mM NaHCO₃, 5 mM KCl, 1 mM KH₂PO₄, 1 mM MgSO₄, 30 mM glucose and 2.5 mM CaCl₂) at 37°C and bubbled with 95% oxygen and 5% carbondioxide. One end of the preparation was connected with a siliconized silk suture to a Grass FT03 force-displacement transducer. An initial force of 10 mN was applied. The phrenic nerve was placed on a bipolar platinum electrode and was stimulated with rectangular pulses of 0.2 ms duration at 20 s (0.05 Hz) intervals at a supramaximal voltage, using a Grass S88 Stimulator. Contractions were recorded on a four channel Grass 79D recorder.

After the development of stable contractions, an appropriate single dose of a neuromuscular blocking agent was added to each bath to produce inhibition of contractions to approximately 5-10% of baseline values after 20 min contact time (this concentration was found to be 3.1 µM for rocuronium bromide). Increasing amounts of reversal agent were then administered into the bath at intervals of 10 min. The % maximum reversal was established. At the end of the experiment, the measured muscle contractions force was plotted against the concentration of reversal agent, and using regression analysis techniques, the 50% reversal concentration was calculated.

After induction of neuromuscular block by rocuronium, the % maximum reversal produced by the addition of a number of γ-cyclodextrin derivatives (compounds **11-16**), a cyclic octasaccharide comprising 4 rhamnosyl-mannopyranosyl units (compound **24**), or a number of paracyclophane derivatives (compounds **17-21** and **23**) are presented in Table II. The results demonstrate that the neuromuscular blocking action of rocuronium can be efficiently

blocked by chemical chelators of variable structure, i.e. by the γ -cyclodextrins 13, 14 and 15, by a cyclic oligosaccharide composed of rhamnose and mannose (24), and by action of the cyclophanes 18 and 23.

TABLE II Mouse hemidiaphragm: maximum reversal (%)

Compound	3.1 μ M rocuronium ¹
CYCLODEXTRINS	
<u>11</u> : γ -cyclodextrin-phosphate sodium salt (DS=3)	34 (360)
<u>12</u> : γ -cyclodextrin-phosphate sodium salt (DS=7)	17 (360)
<u>13</u> : carboxymethyl- γ -CD (DS=3.2)	88 (180)
<u>14</u> : carboxyethyl- γ -CD (DS= 3.8)	87 (144)
<u>15</u> : γ -cyclodextrin (γ -CD)	94 (144)
<u>16</u> : 2-hydroxypropyl- γ -CD (DS= 4)	71 (360)
CYCLIC OLIGOSACCHARIDE	
<u>24</u> : cyclo[(1-4)- α -L-rhamnopyranosyl-(1-4)- α -D-mannopyranosyl]tetraoside	86 (288)
CYCLOPHANES	
<u>17</u> : 1,7,21,27-tetraoxa-9,17,29,37-tetracarboxy-[7.1.7.1]paracyclophane	11 (360)
<u>18</u> : 1,10,24,33-tetraoxa-12,20,35,43-tetracarboxy--[2.2.1.2.2.1]paracyclophane	61 (288)
<u>19</u> : N, N', N'', N'''-tetrakis(carboxyacetyl)-1, 7, 21, 27-tetraaza[7.1.7.1] paracyclophane	2.4 (108)
<u>20</u> : N, N', N'', N'''-tetrakis(4-carboxybutyryl)-1, 7, 21, 27-tetraaza[7.1.7.1] paracyclophane	8.7 (72)
<u>21</u> : N, N', N'', N'''-tetrakis(3-carboxypropionyl)-1, 7, 21, 27-tetraaza[7.1.7.1]para cyclophane	0 (360)
<u>23</u> : N, N', N'', N'''-tetrakis (3-carboxypropionyl)-3,4,5,6,7,8,26,27,28,29,30,31-dodecahydro-1,10,24,33-tetraaza[2.2.1.2.2.1]paracyclophane	90 (58)

¹: concentration of chemical chelator at maximum reversal in μ M in parenthesis;

Example 7.**Calixarene derivatives.**

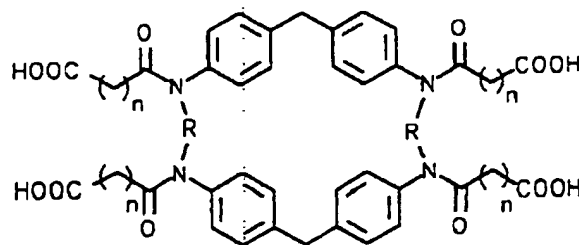
4-Sulfonic calix[6]arene and 4-sulfonic calix[8]arene were obtained from Aldrich.

Reversal of neuromuscular block *in vivo*, induced by rocuronium bromide, was carried out as described in Example 5. The dose (ED_{50}) of the calixarene derivative producing 50 % reversal of steady-state neuromuscular block in the anaesthetised guinea pig was found to be 5.1 $\mu\text{mol/kg}$ for the 4-sulfonic calix[6]arene and 34 $\mu\text{mol/kg}$ for the 4-sulfonic calix[8]arene.

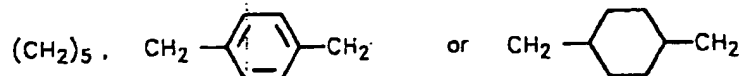
Reversal of neuromuscular block *in vitro* was carried out using the mouse hemidiaphragm preparation as described in Example 6. After induction of neuromuscular block (95 % block) with rocuronium bromide (3.6 μM in the bath) maximum reversal of 124 % and 120 % were recorded for 4-sulfonic calix[8]arene and the 4-sulfonic calix[6]arene, respectively, while the 50% reversal concentrations were found to be 36 μM and 34 μM .

Claims.

1. Use of a chemical chelator for the manufacture of a medicament for the reversal of drug-induced neuromuscular block.
2. The use according to claim 1, wherein the chelator is selected from the group consisting of cyclic oligosaccharides, cyclophanes and calixarenes.
3. The use according to claim 2, wherein the chelator is a cyclic oligosaccharide.
4. The use according to claim 3, wherein the cyclic oligosaccharide is a cyclodextrin or a derivative thereof.
5. The use according to claim 4, wherein the cyclodextrin is γ -cyclodextrin or a derivative thereof.
6. The use according to claim 2, wherein the cyclophane has the general formula A

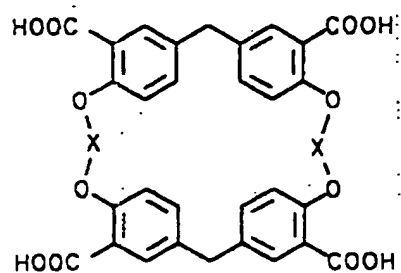


wherein R is

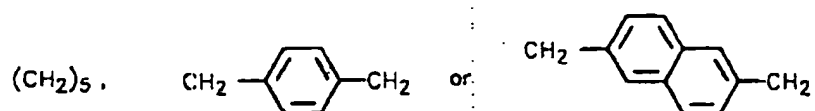


and n is 1-5; or

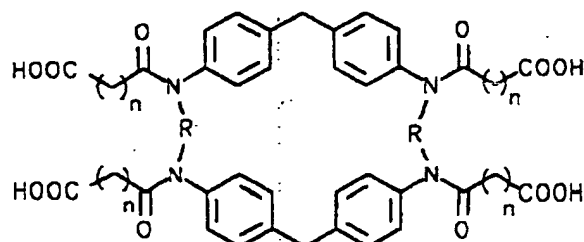
the general formula B



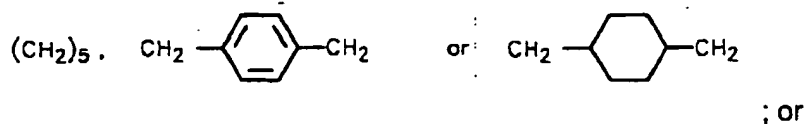
wherein X is



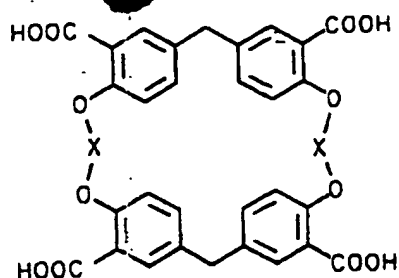
7. The cyclophane derivative having the general formula A



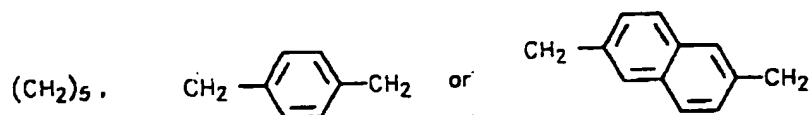
wherein R is



the general formula B



wherein X is



or a pharmaceutically acceptable salt thereof.

8. A kit for providing neuromuscular block and its reversal comprising (a) a neuromuscular blocking agent, and (b) a chemical chelator capable of forming a guest-host complex with the neuromuscular blocking agent.
9. The kit according to claim 8, wherein the neuromuscular blocking agent is selected from the group consisting of rocuronium, vecuronium, pancuronium, rapacuronium, mivacurium, (cis)atracurium, tubocurarine and suxamethonium, and wherein the chelator is selected from the group consisting of cyclic oligosaccharides, cyclophanes and calixarenes.
10. The kit according to claim 9, wherein the neuromuscular blocking agent is rocuronium and the chemical chelator is γ -cyclodextrin or a derivative thereof.
11. A method for reversal of drug-induced neuromuscular block in a patient which comprises parenterally administering to said patient an effective amount of a chemical chelator capable of forming a guest-host complex with said drug.

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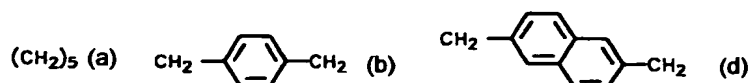
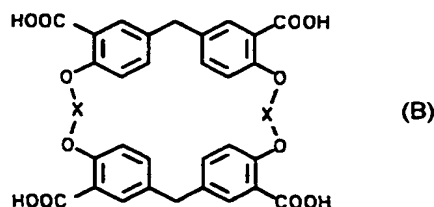
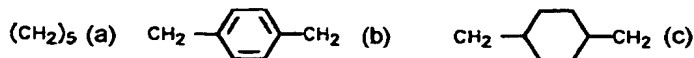
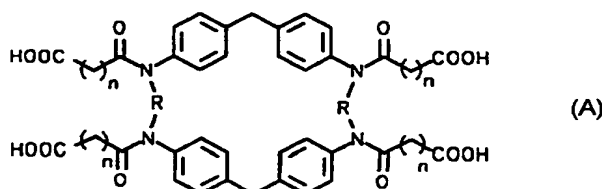
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(54) Title: USE OF CHEMICAL CHELATORS AS REVERSAL AGENTS FOR DRUG-INDUCED NEUROMUSCULAR BLOCK



(57) Abstract: The invention relates to the use of chemical chelators for the preparation of a medicament for the reversal of drug-induced neuromuscular block, to a kit for providing neuromuscular block and its reversal, and to cyclophane derivatives having general formula (A) wherein R is (a), (b) or (c); or general formula (B) wherein X is (a), (b) or (d), or a pharmaceutically acceptable salt thereof.



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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	B. DESIRE: "Interaction of soman with beta-cyclodextrin" FUNDAMENTAL AND APPLIED TOXICOLOGY, vol. 7, no. 4, 1986, pages 647-657, XP000911170 the whole document ---	1-5,8,11
Y	AU 36628 95 A (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 9 May 1996 (1996-05-09) the whole document --- -/--	1-5,8-11

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